ACTA BIOL. SZEGED. 35. PP. 71—80 (1989)

INVESTIGATION OF THE BIOPOLYMER ORGANIZATION OF PARTIALLY DEGRADED EXINES WITH THE FRAGMENTATION METHOD

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(Received: February 27, 1989)

Abstract

Pollen grains of *Alnus glutinosa* (L.) GAERTN. were partially degraded by ten different kinds of experiments. The partially degraded exines were fragmented and the residues were investigated with transmission electron microscope. On the basis of our first results, the advantages and the limits of this method are discussed in this paper. Advantages: There are opportunities to study the different levels of the sporoderm organization in the dimension of the molecules. 2. The biopolymer basic-units could be investigated in the entire exine structures. 3. Highly organized biopolymer structures of different morphology were also to be observed: globular and network structures, mostly built from these, respectively filamentous units, too. The latter ones might be well interpreted by the quasi-crystalloid character of the basic-units of the biopolymer skeleton. Limits: In some cases at interesting or peculiar TEM biopolymer structures there may be doubts concerning their origin.
Key words: Palynology, sporopollenin, biopolymer organization, fragmentation method.

Introduction

Relatively few papers deal with the biopolymer structure of the partially degraded sporoderm (e. g.: ROWLEY, 1975; ROWLEY and PRIJANTO, 1977; ROWLEY et al., 1980; SOUTHWORTH, 1985, 1986; KEDVES, 1988a, b). The ultra-thin sections of the partially degraded sporoderm were investigated with transmission electron-microscope. Later, the observed regular pentagonal polygon basic units were investigated with the modified Markham rotation method (cf. KEDVES et al., 1989). In this case this method was not only verificatory for symmetry. The importance of the problem investigated, and in some place its novel character need to elaborate new or adapt other methods of different fields. The new combination of methods represent one part of this attempt, with the first results.

Material and Methods

The objects of our investigations: pollen grains of *Alnus glutinosa* (L.) GAERTN., were collected on 25 February 1989 in the Botanical Garden of the A. J. University, by Dr. K. MARGÓCZY. After collection the fresh material was frozen at -20 °C to eliminate the alternations, which may happen in consequence of

the autoxidation character of the sporopollenin. For the partial degradation of the exine the following experiments were applied:

235. — 20 mg pollen + 1 ml 2-aminoethanol, temperature 30 °C, length of time 24^h.

236. — 20 mg pollen + 1 ml 2-aminoethanol, temperature 30 °C, length of time 24^h + 10 ml KMnO4 aq. dil., 1%, temperature 30 °C, length of time 24^h.

237. — 20 mg pollen + 1 ml 2-aminoethanol, temperature 30 °C, length of time 24^h + 10 ml KMnO4 aq. dil. 1%, temperature 30 °C, length of time 48^h.

238. — 20 mg pollen + 1 ml 2-aminoethanol, temperature 30 °C, length of time 24^h + 100 ml KMnO4 aq. dil. 1%, temperature 30 °C, length of time 24^h + 2 ml acetic acid anhydride, temperature 30 °C, length of time 24^h.

239. — 20 mg pollen + 1 ml 2-aminoethanol, temperature 30 °C, length of time 24^h + 10 ml KMnO4 aq. dil. 1%, temperature 30 °C, length of time 24^h + 5 ml methanol, temperature 30 °C, length of time 24^h.

The pollen material of the experiments No 250—254 were heated at 100 °C during one hour before the solvent and oxidizing process described formerly. This is the only difference between the two series of experiments. The samples of the experiments No 240—249 were not investigated with the fragmentation method. After the partial degradation of the pollen grains the residues were washed in distilled water. The fragmentation was made with a magnetic stirrer in watered medium, during 30 minutes. The fragmented exines were dropped on a grid cowered with collodium pellicle and then dried. The electron microscopical investigations were made on a Tesla BS-500 transmission electron microscope, resolution 6 Å. The modified Markham rotation method was applied at the TEM pictures where the experiments discovered well the basic, regular pentagonal polygon biopolymer units.

Results

Our results are presented in the order of the experiments: 235. — It is surprising that no matter for evaluation was found after this experiment.

235. — (Plate I, fig. 1—5, plate II). In contrast to the previous one we obtained data for attention, which are different in character. Besides disclosing the basic biopolymer skeleton of regular pentagonal polygon units its highly organized level may be pointed out at this experiment. We observed filamentous units too, which are 25—35 Å large (Plate I, fig. 1, 3) on which occur fragments of darker electron dense biopolymer systems (Plate I, fig. 1, marked with an arrow). The basic pentagonal polygon system is partially larger than those recognized previously

Plate I

1—7. *Alnus glutinosa* (L.) GAERTN.

1. Experiment, No 236, filamentous units on it with regular pentagonal polygon biopolymer units, with strong electron density, marked with an arrow, x150000.

2. Experiment, No 236, the highly organized biopolymer units forming a network. Well shown is the pentagonal polygon basic-skeleton, and the spherical units, which built them, x150000.

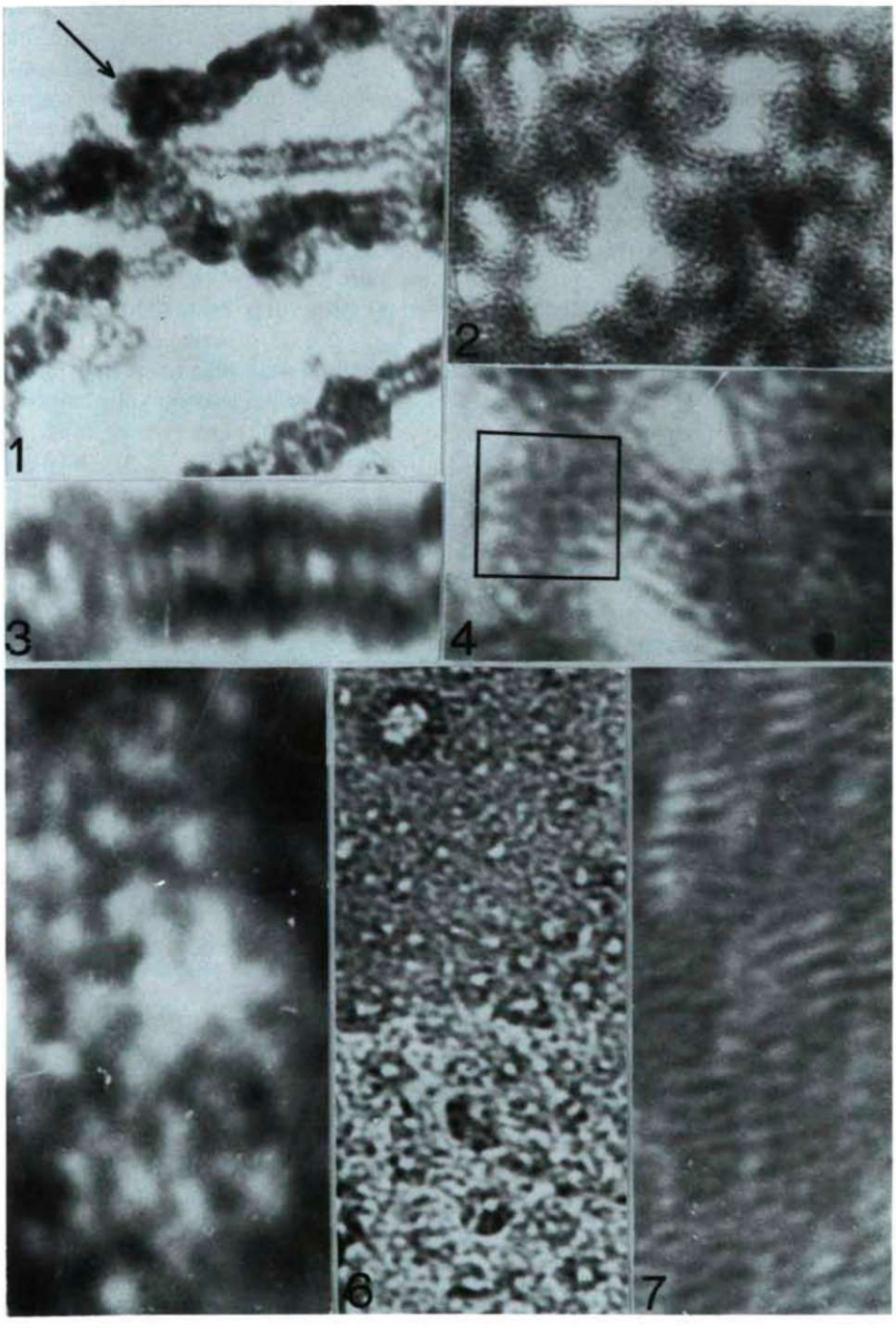
3. Experiment, No 263, detail from the filamentous unit. x500000.

4. Experiment, No 236, detail from the highly organized biopolymer structure forming a network. The central biopolymer unit, which is surrounded with further ones is framed, x500000.

5. Experiment, No 236, detail from the basic biopolymer skeleton, x500000.

6. Experiment, No 237, fragment of doubtful origin, its ultrastructure may be studied on two levels. The darker part is the outer, the bleacher one represents the inner structure, x250000.

7. Experiment No 237, helical structures from a vegetal fragment of doubtful origin, x500000.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(Plate I, fig. 5); 10—20—26 Å. However, the diameters of the highly organized, partially spherical units are essentially identical with the previously published average values (8—12 Å). In some cases one basic, regular pentagonal polygon in central position was observed, this is surrounded by further similar pentagonal biopolymer units (Plate I, fig. 4, plate II, the framed part of the picture). The diameter and the shape of the holes vary; 25—30 Å, mostly isodiametric, but longer, and larger holes (e. g.: 160 Å) were also observed.

237. — This experiment resulted unusual results, in connection with these it is possible that these ultrastructural elements are not of exine origin but other kind of tissue fragment. On the other hand it is important in methodical respect, that this method is suitable to study the submicroscopic structures of the different space levels of the fragments. On picture 6 of Plate I, the darker superficial, and the light-coloured inner part are well shown. Helical structures were also aberved (Plate I, fig. 7), whereas independent of its doubtful origin we belive that they are important.

238. — This experiment well disclosed the basic biopolymer units, the diameter of the regular pentagonal polygon units are in general 8—12 Å. The highly organized spherical units and their arrangement, and the holes between the biopolymer system are similar to those discussed at experiment No 236 (Plate I, fig. 2, plate II).

239. — On the TEM pictures we have observed microscopical fungi, in this way we keep this experiment not appreciable. But it is noteworthy that in some places the basic-biopolymer units of the sporopollenin are well shown.

250. — Similarly to experiment No 235 resulted in no appreciable data.

251. — At this experiment highly organized spherical units, forming a network were observed. In some places the spherical units may be arranged into filaments.

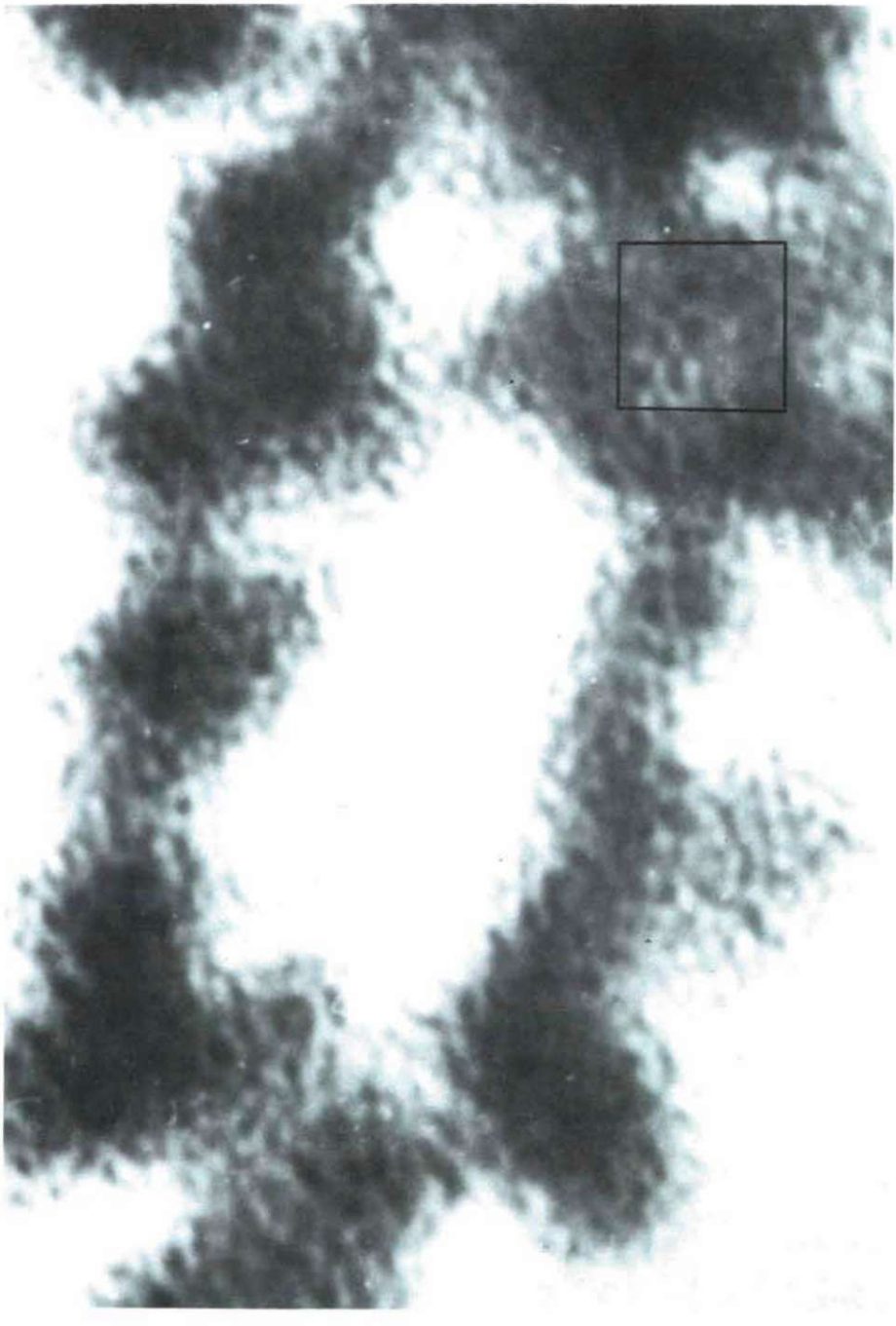
252. — Essentially this experiment gave the same result as at those of No 237 (cf. Plate I, fig. 6).

253. — This experiment resulted in very useful data in the knowledge of the basic biopolymer units of the sporopollenin. In several parts of the TEM pictures we have observed regular pentagonal polygon units and their arrangements too. The modified Markham rotation method was also applied at two biopolymer basic units (Plate IV, fig. 1—4).

Plate II

Alnus glutinosa (L.) GAERTN.

Experiment No 236, the biopolymer organization of the sporoderm is well represented on three levels. The basic biopolymer units are arranged into spherical ones (see the framed part of the picture), and these form also a network, x500000.



254. — This experiment served very appreciable data to the different phases of this kind of method, applied for the first time at this research program. On fig. 2 of Plate V, the different steps of the degradation are well shown until the dissolution. At the last phase the stratification of the exine may not be recognized. On Plate IV, and in picture 1 of Plate V, the morphological characteristic features of the pollen grains of *Alnus* are well shown inside this the biopolymer organization of the sporopollenin, too. The basic biopolymer units may be seen in particular in the tectum, the highly organized ones mostly in the infratectal layer. In the picture of Plate IV, well shown is the dissolution of the exine, and the biopolymer units which emerged into the surrounding medium.

Discussion

The method of the fragmentation, besides the previously used ones, assured new opportunities in the investigation of the biopolymer systems of the partially degraded exines. We need to emphasize that this method completes and not replaces the previously applied ones. We must stress again that in some cases the origin of the biopolymer structures may be doubtful. But this does not diminish the importance of the newly applied method. Resuming, this research direction supports as well the importance of the multidisciplinary investigations.

Plate III

1—4. *Alnus glutinosa* (L.) GAERTN.

1. Experiment, No 253, the regular pentagonal polygons of the basic-biopolymer skeleton are well shown, x500000.

2. Experiment, No 253, detail from the regular pentagonal polygon, basic biopolymer structure, x1 million.

3, 4. Experiment, No 253, the biopolymer structure after the modified Markham rotation, C.P.5.A.5.5., x1 million.

Plate IV

Alnus glutinosa (L.) GAERTN.

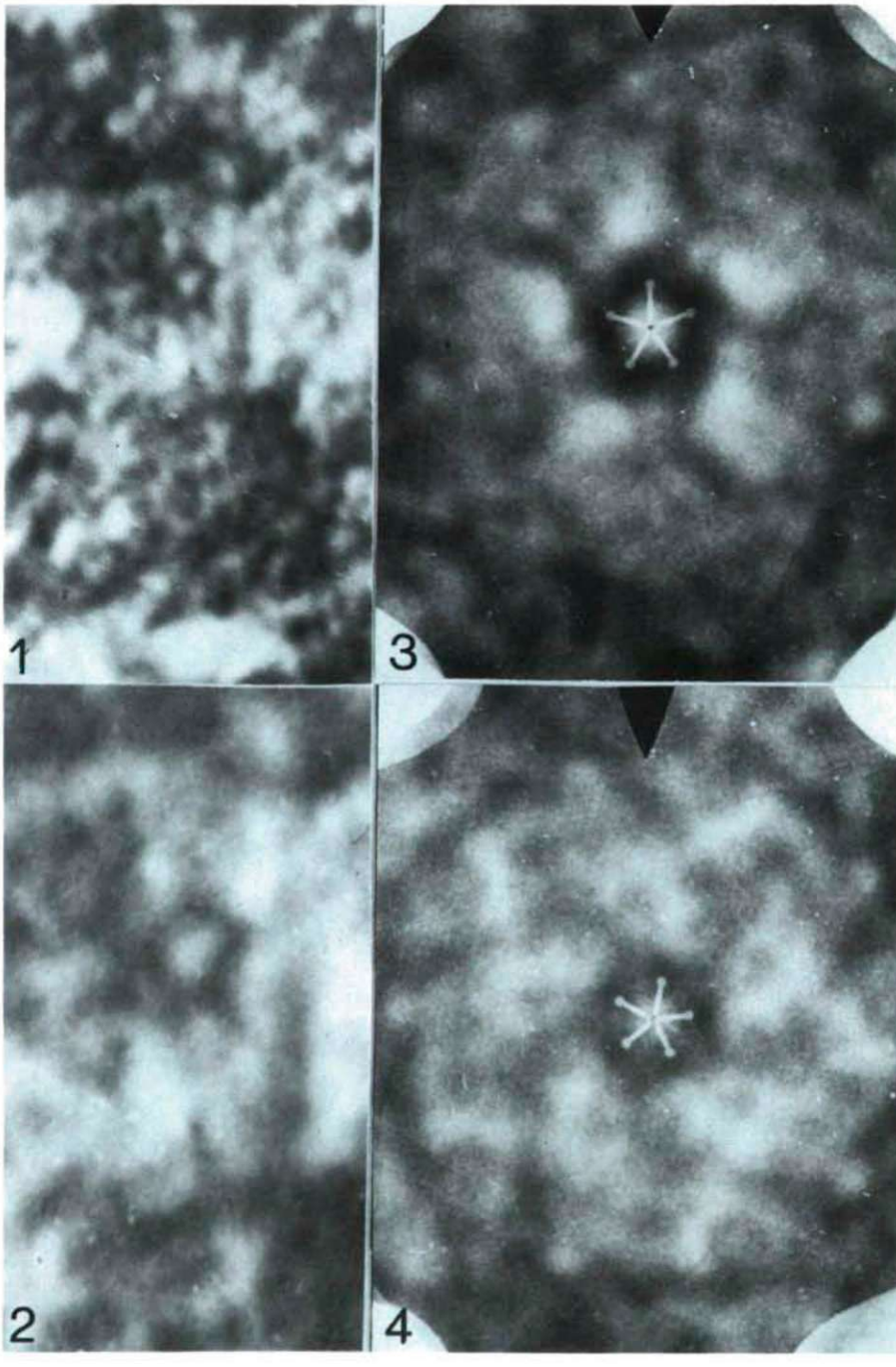
Experiment, No 254, partially degraded exine of the pollen grain, which was originally oriented in equatorial position. The regular pentagonal polygon basic units of the tectum are well shown, the degradation of the surface, and the highly organized biopolymer units of the infratectum, x250000.

Plate V

1—3. *Alnus glutinosa* (L.) GAERTN.

1. Experiment, No 254, detail from the biopolymer organization of the tectum, and the infratectum, x500000.

2, 3. Experiment, No 254, the degrees of fragmentation and partial degradation are well shown, x3600.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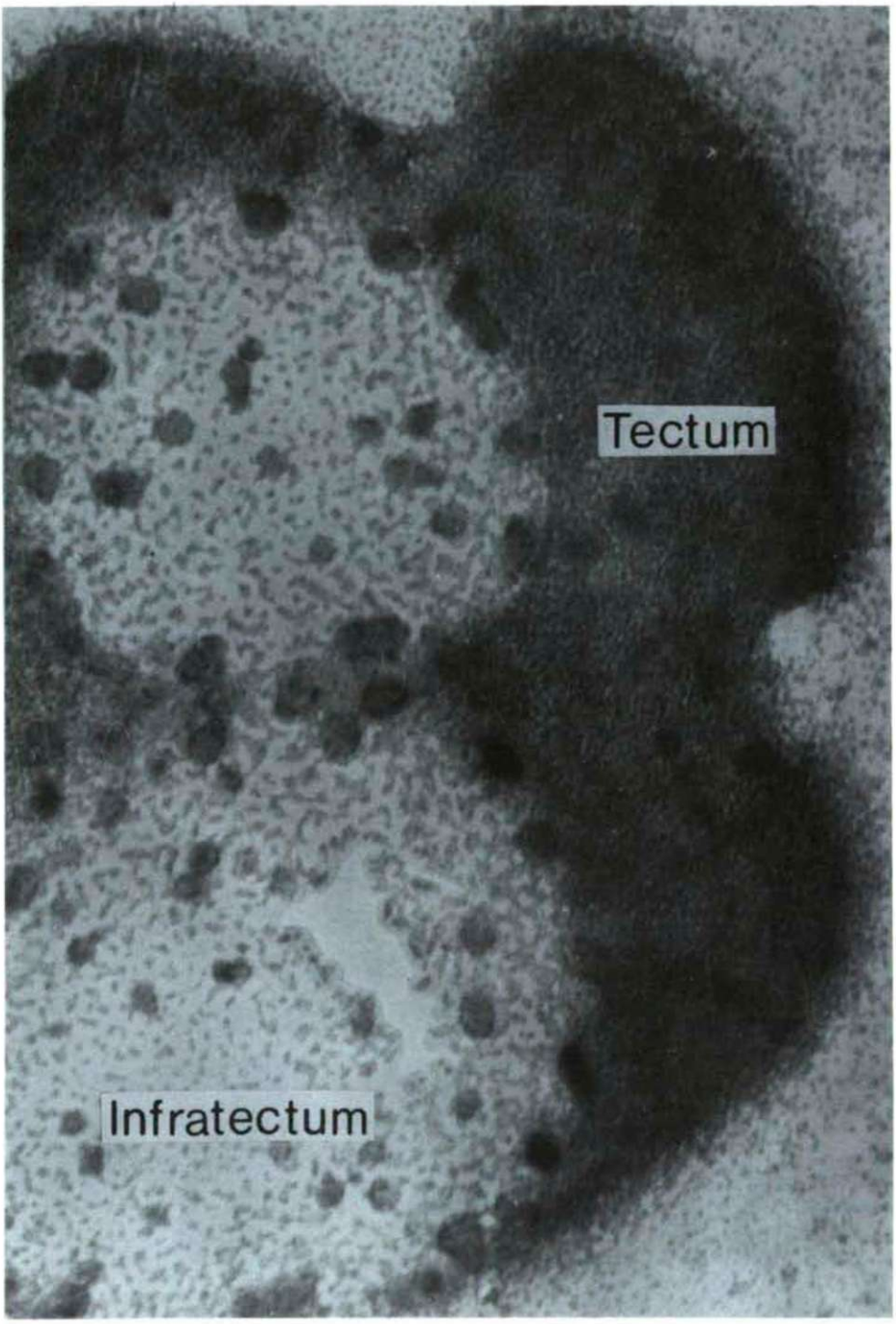
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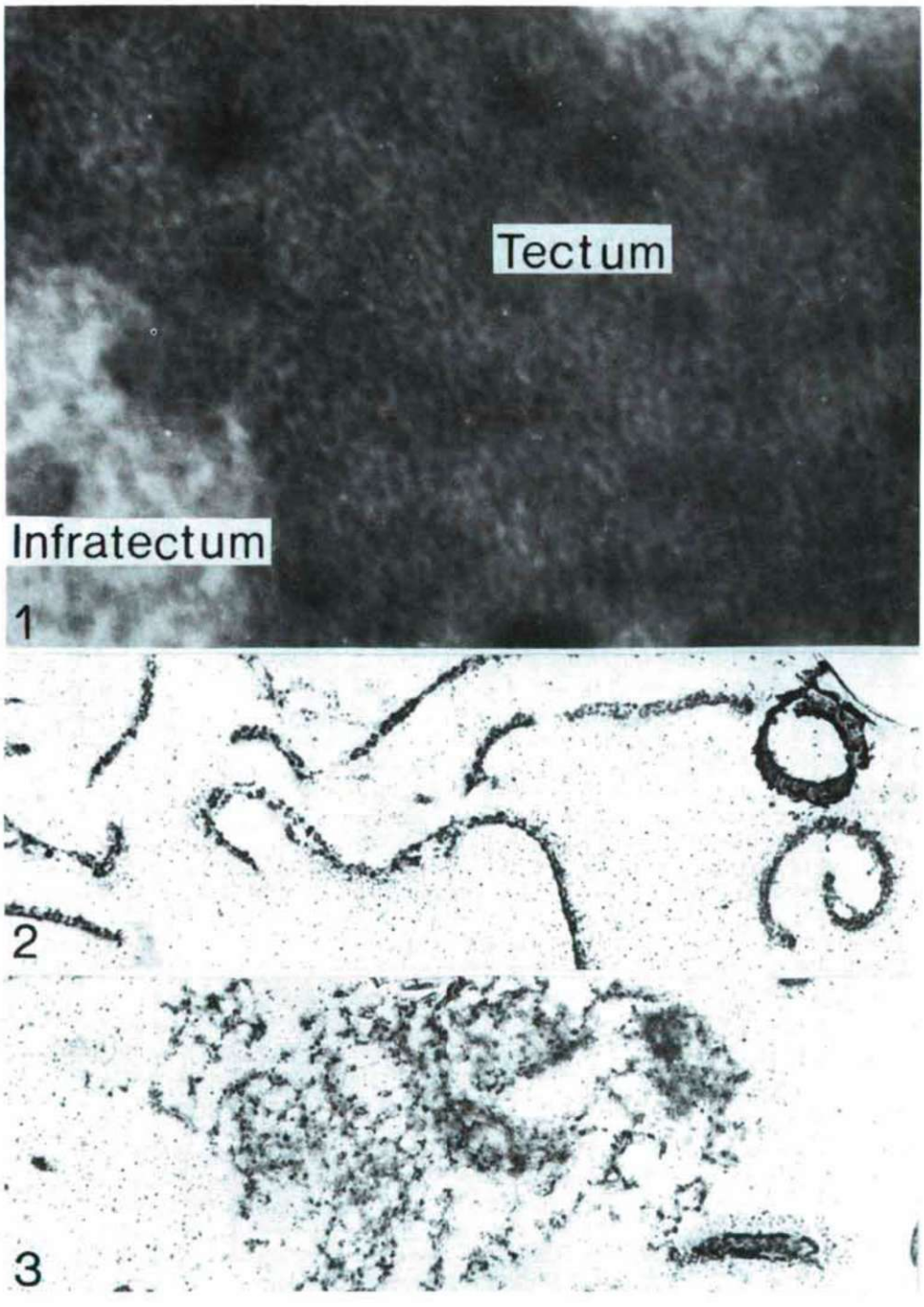
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Acknowledgements

This work was supported by the grant OTKA II—24/88.

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